CHANGES IN THE ALKALI RESERVE, SUGAR CONCENTRATION, AND LEUKOCYTES OF THE BLOOD IN EXPERIMENTAL INFECTIONS

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A previous study of the changes in the leukocytes and the alkali reserve of the blood with experimental infections in rabbits demonstrated that the intravenous injection of suspensions of living pathogenic bacteria is followed within 1 to 2 hours by a leukopenia and a diminution of the alkali reserve of the whole blood.[1] The rapidity and the degree of lowering of the alkali reserve in these experiments seemed to depend on the pathogenicity of the bacteria used. Following depression of the alkali reserve and the leukopenia there is an 18 to 24 hour interval during which the leukocytes increase rapidly in number, and the alkali reserve returns to, or slightly exceeds, the value originally determined for the blood of the rabbit. When the alkali reserve remains depressed the leukocytosis persists, but later when it approaches or exceeds slightly the normal the number of leukocytes also becomes normal. In experiments in which the infection does not subside with such simple and prompt changes, there are irregular depressions of the alkali reserve and coincident periods of leukocytosis until both remain normal, and there is no further evidence by these examinations of disease in the animal. The suggestion is made, on the basis of these results, that lowering the alkali reserve of the blood sufficiently, or the factors associated with its depression, are concerned with the production of a general leukocytosis.

An excellent summary of the theories advanced to explain generalized leukocytosis was given by Gehring.[2] Following Metschnikoff's studies of the activities of leukocytes and their highly important function in immunity, the value of artificially produced leukocytosis in combating infectious diseases was generally recognized. At first, variations in the number of leukocytes of the blood were thought to occur only in the peripheral vessels, but later this

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[1] Hirsch: Jour. Infect. Dis., 1921, 28, p. 275.

[2] Ztschr. exper. Pah., 1915, 17, p. 161.

was disproved. From the earliest studies, the theories concerning the origin of leukocytosis included the notion of some chemical substance attracting the leukocytes into the circulation from reserve places or stimulating their formation in the hematopoietic tissues. Many experiments in vitro demonstrated chemical substances with a positive chemotaxis toward leukocytes but all these are inconclusive as regards a satisfactory explanation of a generalized leukocytosis.

A further step in explaining generalized leukocytosis was made by demonstrating that toxins stimulate the leukocyte-forming tissues such as bone marrow. Other chemical agents, such as a toxin acting on the smooth muscle of the spleen and lymph nodes, mechanically forcing the cells contained into the circulation (lymphocytosis) and stimulation of the hematopoietic tissues by the disintegration products of red blood corpuscles, have been given as causes of leukocytosis. The leukocytosis of hemorrhage was thought to result from some indirect stimulus of the bone marrow, and other substances such as the brokendown products of leukocytes themselves were considered for a time as causing leukocytosis.

The confusion in explaining leukocytosis was increased by observing leukocytosis after the action of certain physical agents on the body, such as massage, heat or cold, trauma, muscular activity, chemical irritation of the skin, psychic stimuli, pregnancy, etc., also leukocytosis with digestion.

Pepper and Miller[3] in studying the leukopenia and subsequent leukocytosis in rabbits after injections of both living and killed typhoid bacilli, could establish no relationship between leukocytosis and the content in the urine of the total nonprotein urea, and allantoin nitrogen, all end-products of nucleic acid metabolism which these authors thought might be correlated with changes in the number of leukocytes of the blood. This briefly summarizes what is at present contained in the literature to explain generalized leukocytosis.

With the changes mentioned in the alkali reserve of the blood, there are without doubt others as yet unsuspected and of a chemical nature, since alterations in H-ion concentration are known to accompany or bring about disturbances in the composition and properties of such an intricate solution of colloids and crystalloids as blood plasma. One of these changes, it was fair to suppose, concerns the dextrose of the blood, and some hint of changes in the amount of glucose in the blood with infections is found in the report of Rohdenberg and Pohlman[4] who consider the hyperglycemia of animals immunized against bacteria an index of the degree of immunization. Elias[5] observed hyperglycemia and glycosuria in rabbits and dogs following the feeding of dilute hydrochloric acid, while even earlier writers, such as Pavy in 1864, Goetz in 1867, and Naunyn in 1868[6] had

[3] J. Infect. Dis., 1916, 19, 694.

[4] Am. J. Med. Sc., 1920, 159, p. 853.

[5] Biochem. Ztschr., 1913, 48, 120.

[6] Kulz, E.: Beiträge zur Lehre vom künstlichen Diabetes, Arch. ges. Physiol., 1881, 24, p. 97.

observed glycosuria following the feeding or injection of phosphoric, lactic, or hydrochloric acids. Underhill,[7] commenting on the effects of acidosis on carbohydrate metabolism, says the introduction of acid into the organism is associated with a disturbance of blood sugar content and is manifested in one direction; namely, blood sugar content is augmented. Other writers have made similar comments.[8]

With these observations as a basis, an experimental study in rabbits was made to determine the effect on the blood sugar concentration of

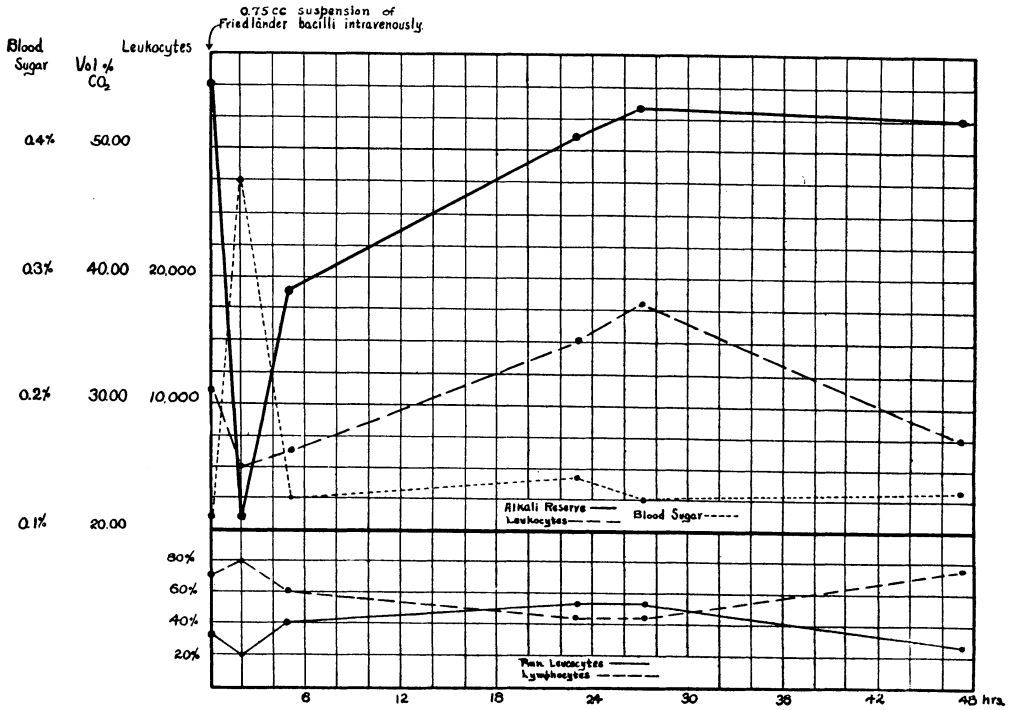

Chart 1.—Effect of injection of living pathogenic bacteria.

intravenous injections of suspensions of living pathogenic bacteria, paralleled by estimations of the alkali reserve of the whole blood, and by determinations of the number of leukocytes. The following bacteria were used: B. typhosus, B. paratyphosus A, B. paratyphosus B, B. dysenteriae (Flexner), B. Friedländer, B. coli, streptococcus hemolyticus, Pneumococcus, and B. welchi. The bacteria were grown on

[7] Jour. Biol. Chem., 1916, 25, p. 463.
[8] Mathews, A. P.: Physiological Chemistry, 1915, p. 247.

RESULTS OF EXPERIMENTS WITH BACTERIA AND WITH INJECTIONS OF GLUCOSE AND ACID POTASSIUM PHOSPHATE SOLUTIONS

Hour	B. typhosus			Pneumococcus			Hemolytic Streptococcus			Bacillus welchii			Dextrose 3¾ Gm. Subcutaneously			Monobasic Potassium Phosphate, 15 C c 7% Sol. (80 C c N/10 HCl)			Monobasic Potassium Phosphate, 15 C c 7% Sol. (80 C c N/10 HCl)		
	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes
0	68.23	0.120	13,000	60.23	0.124	10,000	59.35	0.106	8,000	53.52	0.109	8,700	54.83	0.10	9,000	53.02	0.117	11,000	61.72	0.113	8,000
1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	49.40	0.224	15,600	.....	.....	.....	46.17	0.121	6,600
2	53.52	0.198	2,800	52.77	0.133	4,500	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	41.56	0.248	5,900
3	.....	.....	.....	.....	.....	.....	54.64	0.121	5,000	48.05	0.157	2,000	.....	.....	.....	43.98	0.134	10,500	32.44	0.240	dead
4	47.08	0.200	2,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	.....	.....	.....	43.50	0.138	4,500	54.64	0.134	5,000	.....	.....	.....	40.49	0.224	15,600	.....	.....	.....	.....	.....	.....
6	47.08	0.150	4,400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	43.20	0.133	15,500	.....	.....	.....
22	.....	.....	.....	.....	.....	.....	.....	.....	.....	56.25	0.110	14,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	47.46	0.110	30,000	54.80	0.123	7,000	63.27	0.120	9,800	.....	.....	.....	51.37	0.100	15,000	.....	.....	.....	.....	.....	.....
24	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	58.63	0.113	14,800	.....	.....	.....
28	44.39	0.096	19,600	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29	.....	.....	.....	52.77	0.125	8,200	58.59	0.141	9,300	46.17	0.114	17,500	.....	.....	.....	.....	.....	.....	.....	.....	.....
46	.....	.....	.....	.....	.....	.....	.....	.....	.....	45.78	0.118	9,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
47	.....	.....	.....	50.39	0.152	6,600	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2 days	59.90	0.150	20,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	10,000	.....	.....	.....	.....	.....	.....
3 days	67.20	0.113	25 000	44.86	0.152	11.000	.....	.....	.....	51.21	0.125	10,500	.....	.....	.....	.....	.....	10,800	.....	.....	.....
4 days	64.51	0.149	15,600	50.18	0.142	12,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5 days	.....	.....	.....	53.81	0.111	9,600	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6 days	.....	.....	.....	59.04	0.153	9.600	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

Hour	B. Paratyphosus "A"			B. Paratyphosus "B"			B. dysenteriae (Flexner)			B. coli			B. diphtheriae			B. coli			B. coli		
	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes	Alkali Reserve	Blood Sugar, Percentage	Leukocytes
0	51.26	0.114	11,700	59.61	0.115	6,400	59.04	0.153	9,500	58.00	0.113	8,500	49.67	0.132	16,900	60.34	0.145	8,200	43.01	0.153	8,200
1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	44.91	0.152	14,300	.....	.....	.....	.....	.....	.....
2	.....	.....	.....	.....	.....	.....	.....	.....	.....	53.37	0.160	1,300	.....	.....	.....	41.87	0.330	2,100	16.12	0.412	3,500
3	45.66	0.165	2,700	52.23	0.160	4,000	47.82	0.246	3,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	dead	.....	.....
4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	41.29	0.143	10,800	46.51	0.212	2,100	.....	.....	.....
5	.....	.....	.....	.....	.....	.....	34.84	0.208	3,100	44.91	0.124	3,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	43.01	0.111	33,400	52.23	0.114	10,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	42.45	0.152	4,000	.....	.....	.....
22	.....	.....	.....	.....	.....	.....	44.99	0.099	20,000	.....	.....	.....	56.10	0.105	40,200	.....	.....	.....	.....	.....	.....
23	.....	.....	.....	.....	.....	.....	.....	.....	.....	57.95	0.102	15,700	.....	.....	.....	dead	.....	.....	.....	.....	.....
24	39.22	0.080	47,000	57.53	0.099	56,500	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
27	.....	.....	.....	.....	.....	.....	46.84	0.105	41,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	57.07	0.123	15,100	.....	.....	.....	.....	.....	.....
29	.....	.....	.....	.....	.....	.....	.....	.....	.....	52.40	0.116	21,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
30	57.05	0.101	50 000	54.82	0.107	42,000	.....	.....	53,000	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
46	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	49.77	0.142	23,500	.....	.....	.....	.....	.....	.....
47	.....	.....	.....	.....	.....	.....	.....	.....	.....	54.36	0.136	13,200	.....	.....	.....	.....	.....	.....	.....	.....	.....
2 days	64.57	0.124	23.400	58.14	0.116	14,500	dead	.....	.....	.....	.....	.....	50.65	0.116	13,400	.....	.....	.....	.....	.....	.....
3 days	59.96	0.128	15.800	57.17	0.131	6,000	.....	.....	.....	55.22	0.143	21,100	63.60	0.119	9,800	.....	.....	.....	.....	.....	.....
4 days	.....	.....	.....	.....	.....	.....	.....	.....	.....	49.26	0.112	7,600	.....	.....	.....	.....	.....	.....	.....	.....	.....

plain or blood-agar slants in pure culture for 14-48 hours, and varying fractional amounts depending on their pathogenicity in rabbits were injected intravenously in 0.75 to 2.0 c c volumes of sterile normal salt solution. The alkali reserve, the sugar concentration, and the number of leukocytes of the blood were determined before, and at, intervals after the injections, the amount of blood taken from the ear veins for each set of determinations being from 3 to 4 c c. The blood sugar estimations were made according to Folin and Wu [9] with the special tubes recommended, the alkali reserve of the whole blood according to Van Slike and Cullen.[10]

With the initial lowering of the alkali reserve of the blood there is a sudden and transient increase in the concentration of the sugar in the blood which in degree seems to be in proportion with the depression of the alkali reserve. This transient rise in the concentration of the sugar seemingly reaches its maximum with the lowest level to which the alkali reserve of the blood falls and then returns within 2 to 4 hours to the normal concentration even though the alkali reserve remains depressed. The accompanying chart graphically represents this change in an experiment with Friedländer bacilli, and in the table are summarized the results of experiments with other bacteria and those with injections of glucose and acid potassium phosphate solutions. The alkali reserve, the concentration of sugar, and the number of leukocytes were not changed in the blood of rabbits injected intravenously with sterile normal salt solution (10 c c) or subjected only to the handling and bleeding necessary for the experiments.

The injection of acid potassium phosphate solutions depresses the alkali reserve of the blood, and with this there is an increase in the concentration of the sugar in the blood similar to that produced by the injections of bacteria. In other experiments with the injection of these solutions, a leukocyte curve was observed similar to those produced by bacteria.

Having observed depression of the alkali reserve of the blood regularly after injections of pathogenic bacteria in rabbits, attempts were made to prevent this depression by subcutaneous injections of sodium carbonate and bicarbonate solutions. Two equal-sized rabbits received intravenously equal amounts of a typhoid bacillus suspension, one of them subcutaneously 18 c c of a 5% sodium carbonate solution (equiva-

[9] Jour. Biol. Chem., 1919, 38, p. 81. Ibid., Supplement I., 1920, 41, p. 367.
[10] Ibid., 1917, 30, p. 289.

lent to 188 c c 0.1 N NaOH) in divided fractions, the other rabbit 8 c c of sterile normal salt solution. The alkali reserve of the rabbit receiving the carbonate solution decreased from 67.27 carbon dioxide volumes per cent. to 27.86 at the 25th hour when the animal died. The other rabbit lived, and the leukocyte, alkali reserve, and sugar concentration curves of each are similar to those given for typhoid and other pathogenic bacteria. The depression of the alkali reserve in other rabbits injected with colon and Friedländer bacilli could not be prevented by sodium carbonate solutions given subcutaneously.

Differential leukocyte counts were made of the blood of rabbits before and at intervals after the injection of bacteria. In the rabbits examined and regarded as normal the leukocytes of the blood are distributed approximately as follows: lymphocytes—large 10 to 15%, small 35 to 55%; transitional leukocytes, 0 to 1%; mononuclear leukocytes, 0 to 1%; polymorphonuclear leukocytes—neutrophil, 30 to 50%, eosinophil, 0 to 1%, basophil 1 to 6%.

During the early part of the leukopenia stage the percentage of lymphocytes is somewhat greater than that of the polymorphonuclear leukocytes (chart). In a short time the polymorphonuclear ratio rises so that as the circulating white blood cells increase the percentage of neutrophil leukocytes may rise to 80 or 90. Later the lymphocytes form an increasing proportion of leukocytes until finally the normal ratio is again reached. When the number of leukocytes decreases rapidly with the return to normal of the akali reserve, the polymorphonuclear leukocytes seem to disappear first, leaving a high percentage of lymphocytes in the blood. In a number of experiments with the injection of acid phosphate solutions the changes in the number and ratio of leukocytes followed curves similar to those obtained with bacteria.

COMMENT

An increased concentration of sugar in the blood with acidosis is well known. On this basis, then, the occurrence of hyperglycemia with experimental infections seems to depend on a lowering of the alkali reserve (acidosis), and the degree of hyperglycemia on the extent to which the alkali reserve is depressed. Milroy [11] observed a loss of the alkali reserve of the blood after hemorrhage, but reported no studies of the variations in the concentration of the blood sugar.

[11] Jour. Physiol., 1917, 51, p. 259.

Tatum [12] found in rabbits a lowering of the alkali reserve and a hyperglycemia immediately after severe hemorrhage. In 12 to 24 hours the sugar content of the blood returned to normal, and the alkali reserve returned to, or sometimes exceeded, the value determined as normal. Another interesting observation on the relation of lowered alkali reserve of the blood to hyperglycemia was made by Peters and Geyelin [13] who found that the injecton of epinephrin in diabetic patients and in normal human beings was accompanied by a simultaneous diminution of the alkalinity of the blood and a hyperglycemia. They regard decreased alkalinity of the blood as important in producing hyperglycemia of this type. Rohdenberg and Pohlmann [4] make no reference to alkali reserve changes in the blood which presumably resulted from their injections of bacteria.

While the notion that an acidosis accompanies acute infections has been expressed and attempts to correct it have been made by the administration of carbonate solutions, one of the clearest demonstrations of a change in the H-ion concentration of the blood in acute infections has been made recently by Dragstedt.[14] He observed in rabbits infected with hemolytic streptococci a variation in the H-ion concentration of the blood of from P_H 7.65 or 7.75, which is normal, to P_H 7.3 in the infected rabbits shortly before death.

CONCLUSIONS

Depression of the alkali reserve of the blood in rabbits by intravenous injections of pathogenic bacteria is accompanied by a transient hyperglycemia, the degree of hyperglycemia apparently depending on the extent of alkali reserve diminution.

Subcutaneous administration of carbonate or bicarbonate solutions does not prevent the acidosis produced by these injections of bacteria.

Injections of acid potassium phosphate solutions depress the alkali reserve of the blood, this lowered alkalinity being associated with a hyperglycemia and by changes in the number of leukocytes similar to those following injections of bacteria.

The concentration of sugar in the blood seems to be independent of the changes in the number of leukocytes.

[12] Jour. Biol. Chem., 1920, 41, 59.
[13] Ibid., 1917, 31, p. 471.
[14] Jour. Infec. Dis., 1920, 27, p. 452.